PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference P206194PCT MVE	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/NL2004/000017	International filing date *(day/month/year)* 09.01.2004	Priority date *(day/month/year)* 10.01.2003
International Patent Classification (IPC) or national classification and IPC C12Q1/68		
Applicant KEYGENE N.V. et al.		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 8 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 1 sheets, as follows:
      ☐ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the opinion
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☐	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☐	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☐	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
04.08.2004	11.03.2005
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Ulbrecht, M Telephone No. +49 89 2399-7710

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/NL2004/000017

Box No. I Basis of the report

1. With regard to the **language**, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This report is based on translations from the original language into the following language , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3 and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4)
   - ☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages

1-20	as originally filed

Claims, Numbers

2, 3, 5-14	as originally filed
1, 4	received on 17.02.2005 with letter of 17.02.2005

Drawings, Sheets

1/3-3/3	as originally filed

☒ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1,4,7,8
	No:	Claims	2,3,5,6,9-14
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-14
Industrial applicability (IA)	Yes:	Claims	1-14
	No:	Claims	

2. Citations and explanations (Rule 70.7):

see separate sheet

Supplemental Box relating to Sequence Listing

Continuation of Box I, item 2:

1. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this report has been established on the basis of:

   a. type of material:

   ☒ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☐ in written format

   ☒ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed

   ☐ filed together with the international application in computer readable form

   ☒ furnished subsequently to this Authority for the purposes of search and/or examination

   ☒ received by this Authority as an amendment on

2. ☒ In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional observations, if necessary:

Re item V.

1. Reference is made to the following document:
   D1: Genome Research (06-2000), 10(6), 789-807

2.1 D1 teaches a method for providing an integrated genetic and physical map of AFLP markers for a part of the sorghum genome (p. 794, c. 2, § 4 - p. 797, c. 1, § 1; p. 805, c. 2, § 5 - p. 806, c. 1, § 1; Fig. 4-7). Said method comprises providing at least two AFLP markers (= genetic markers) and a library of BAC clones which are distributed into a multitude of pools. From said pools AFLP fingerprints are generated and individual pools which contain an AFLP fragment of interest are identified. Due to the pooling strategy the method further inherently comprises the generation of an AFLP fingerprint for each individual clone in the pool identified above and the identification of the AFLP fingerprint of clones containing the AFLP fragment of interest. The method further comprises the generation of a contig comprising said individual clones. The repetition of the above steps is implicit as further AFLP fragments are physically mapped. The method further links at least two contigs and thereby generates an integrated physical and genetic map which comprises at least two AFLP markers (Fig. 7). The AFLP primers used to obtain the AFLP fingerprints of the pools and of the individual clones always contain 3 selective nucleotides (p. 803, c. 1, § 1 - p. 806, c. 1, § 1). Thus, using the variables of claims 1 and 2, the following applies to the primers used in the method of D1: K, L, M and N are 3 and K+L = M+N. As the method of D1 contains all the steps of the method according to claim 2 and furthermore leads to the physical mapping of AFLP markers , said claim lacks novelty over D1 (Art. 33(2) PCT).

2.2 The uses according to claims 11-14 are disclosed in D1 (supra). Hence, the disclosure of D1 also prejudices the novelty of said claims (Art. 33(2) PCT).

2.3 The additional features suggested by claims 5, 6, 9 and 10 are known from D1 (supra; p. 793, c. 1, § 2 - c. 2, § 1; p. 804, c. 2, § 1; Fig. 2) and thus do not establish novelty over D1 (Art. 33(2) PCT). Also, the features suggested by claim 3 are considered to be implicit in the method of D1 (supra) and thus neither establish novelty (Art. 33(2) PCT).

2.4 The subject-matter of claims 1, 4, 7 and 8 is novel as the combination of features suggested by said claims is not known in the prior art (Art. 33(2) PCT).

3.1 The subject-matter of claim 1 differs from D1 representing the closest prior art in that the sum of selective nucleotides (K+L) of the AFLP primers used to characterise and identify pools positive for the AFLP fragment of interest is larger than the sum of selective nucleotides (M+N) of the AFLP primers used to generate the contig comprising the clone identified to contain the AFLP fragment of interest, i.e. (K+L)-(M+N)>1. To solve the problem of allowing to generate a high resolution physical mapping the selectivity of both primers used to generate the contig has to be lower than that of the primers used in the preceding steps. The definition given by present claim 1, however, also encompasses the possibility of one of the primers used in the contig generation having a decreased selectivity, whereas the other having an increased selectivity, as long as the criterion (K+L)-(M+N)>1 is met. For instance, claim 1 also covers the possibility that K=5, L=5, M=2 and N=7 which would not result in the generation of AFLP fragments allowing the generation of a physical map with higher resolution as compared to the method of D1. Consequently, the foregoing problem is not regarded as being solved and the definition of the selective nucleotides given by claim 1 is considered an arbitrary modification of the method of D1 falling within the scope of routine experimentation of the skilled person which does not bring about any technical surprising effects and which does not establish an inventive step (Art. 33(3) PCT).

3.2 The same considerations also apply to claim 4 (Art. 33(3) PCT)

3.2 The additional features suggested by claims 7 and 8 relate to routine experimentation which do not produce any unforeseeable effects and thus do not establish an inventive step (Art. 33(3) PCT).

4. Industrial applicability of the subject-matter of claim 1-14 is acknowledged (Art. 33(4) PCT).

5.1 As derivable from the description as a whole and in particular from the teaching at p. 15, l. 23 - 30 that the following features are essential to achieve the identification of

clones containing the AFLP fragment of interest (step (g) of claim 1 and step (f) of claim 2) and their integration into a contig (step (h) of claim 1 and step (g) of claim 2).:

(1) The AFLP primers used in the first and second amplification are identical except for a reduced number of selective nucleotides contained in the primers used in the second amplification.

(2) The contig is generated based on similarities of AFLP fingerprints generated by the said second amplification using the said less selective primers.

Since independent claims 1 and 2 do not contain these features they do not meet the requirement following from Art. 6 PCT taken in combination with R. 6.3(b) PCT that any independent claim must contain all the technical features essential to the definition of the invention.

5.2 Claims 1 and 2 relate inter alia to "characterising the genetic markers by means of at least one AFLP fragment" (step (b) of claim 1 and step (a) of claim 2) as well as to "identifying in the multitude of pools a pool in which an AFLP fragment of interest is present".
The term "genetic marker" is not clear and causes problems in construing the scope of said claims (Art. 6 PCT). The only genetic markers taught by the description and used in the claimed method are AFLP markers i.e AFLP fragments. No mention is made of characterising a genetic marker other than said AFLP marker by means of an AFLP fragment. Also, no support can be found for characterising at least two individual genetic markers by means of at least one AFLP fragment as suggested by claim 1. Hence, claims 1 and 2 are not supported by the description (Art. 6 PCT), which does not disclose this feature of the invention in a manner clear and complete for it to be carried out by the skilled person (Art. 5 PCT).
For the purpose of examination claims 1 and 2 were interpreted as referring to the mapping of AFLP markers i.e. AFLP fragments only.
As regards the expression "identifying in the multitude of pools a pool in which an AFLP fragment of interest is present", the use of the undefined article causes problems in construing the scope of said claims (Art. 6 PCT). If said feature related to the identification of a single said pool, the following considerations would apply. The

description only teaches the selection of pools in which said AFLP fragment is present. Consequently, claims 1 and 2 interpreted to relate to the identification of said single pool would not be supported by the description (Art. 6 PCT), which does not disclose this feature of the invention in a manner clear and complete for it to be carried out by the skilled person (Art. 5 PCT).
For the purpose of examination the above term was interpreted as referring to the identification of pools containing the said AFLP fragment in the multitude of pools.

5.3 As AFLP fragments are considered to represent genetic markers (supra), it is not clear to which physical markers is referred to in claim 2, thereby rendering the definition of said claim unclear (Art. 6 PCT). Therefore, it is also not clear which genetic markers are linked to which physical markers. For the purpose of examination the method of claim 2 is construed as referring to the physical mapping of genetic markers, namely AFLP markers.

5.4 The distinction between the technical features referred to by the terms "integration of/integrated genetic and physical map(s)", "linking a genetic marker to physical marker" and "linking genetic and physical genome maps" used in claims 1, 2 and 11-14 is not clear (Art. 6 PCT). It appears that all said expressions relate to the same technical features. Therefore, the subject-matter of claims 11 and 13 is considered indistinguishable rendering one of said claims superfluous (Art. 6 PCT).

5.5 The term "AFLP primer" used in claims 11, 13 and 14 is vague and unclear and leaves the reader in doubt as to the meaning of the technical feature to which it refers, thereby rendering the definition of the subject-matter of said claims unclear (Art. 6 PCT).

5.6 The generic acronym FPC used in claim 9 has no well-recognised meaning and leaves the reader in doubt as to the technical features to which it refers (Art. 6 PCT).

Amended claims

1. A method for providing an integrated genetic and physical map of a genome or part thereof, the method comprising the steps of :

(a) providing at least two individual genetic markers for the genome or part thereof, preferably in the form of a genetic map;

(b) characterising the genetic markers by means of at least one AFLP fragment identified through AFLP fingerprinting ;

(c) providing a library of clones comprising fragments of the genome or part thereof, preferably an artificial chromosome library such as a BAC or YAC ;

(d) generating a multitude of pools, each pool containing a multitude of individual clones from the library;

(e) generating an AFLP fingerprint for each of the pools;

(f) identifying in the multitude of pools a pool in which an AFLP fragment identified in (b) is present in the fingerprint of the pool;

(g) generating an AFLP fingerprint for each of the individual clones in the pool identified in (f), and identifying the clone containing the AFLP fragment identified in (b) in its AFLP fingerprint ;

(h) generating a contig comprising the individual clone identified in step (g);

(i) repeating steps (f-h) for at least a second AFLP fragment identified in (b) whereby the second or further AFLP fragments characterise a second or further genetic marker ; and

(j) linking at least two of the contigs obtained in (h) to thereby obtain an integrated physical and genetic map of the genome or part thereof, which comprises at least two genetic markers; wherein the forward and reverse AFLP primers used in step (b) and (e) comprise K respectively L selective nucleotides at the 3'-end of the primers, wherein the forward and reverse AFLP primers used in step (g) comprise M respectively N selective nucleotides at the 3'-end of the primers, wherein K, L, M, N are integers from 0 to 10, and wherein (K+L)-(M+N) is at least 1.

4. Method according claims 1-3, wherein (K+L)-(M+N) is at least 2, preferably at least 3, more preferably at least 4.